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THE RESPIRATORY RESPONSE TO ASPHYXIA OF *TYPHLOGOBIUS CALIFORNIENSIS* (TELEOSTEI: GOBIIDAE) AND SOME RELATED GOBIES [1]

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Fishes of the large and diverse teleost order Gobiiformes have successfully invaded many habitats characterized by rigorous and fluctuating environmental conditions. In the Californian (warm-and cool-temperate) region of the eastern Pacific are found the nine species of the *Lepidogobius* group. These gobies inhabit, with several exceptions, the shallow waters of estuaries, bays and coastal sloughs. Several species are known to take refuse in the burrow of various substrate-inhabiting invertebrates during tidal exposure, and one, *Typhlogobius californiensis* Steindachner, is an obligate commensal of the burrowing ghost shrimp *Callianassa affinis* Holmes.

Typhlogobius, the blind goby of California, is highly specialized for its unusual mode of life. The pelagic larvae have functional eyes which are overgrown by the skin soon after settling and metamorphosis. They are uncompromising in their choice of a host; adults are never found elsewhere than in *Callianassa affinis* burrows. These burrows are largely confined to the intertidal region along the open coast, in areas of mixed sand and rocks (MacGinitie, 1939).

Tolerance of hypoxic conditions would be of adaptive significance for *Typhlogobius*, since the interstitial water of marine beaches is known to often be deficient in oxygen (Gordon, 1960; Bradfield, 1964). *Callianassa affiinis*, the host of *Typhlogobius*, is extremely tolerant of hypoxic conditions (Thompson and Pritchard, 1969), as are many other littoral burrowing invertebrates. Several authors have noted that *Typhologius* has a remarkable ability to withstand conditions of oxygen depletion which would be rapidly fatal to other fishes (Rosa Eigenmann, 1890; MacGinitie, 1939).

Other related gobies are found in the shallow muddy bays and sloughs of southern California. One of these, *Gillichthys mirabilis* Cooper, is known to take refuge in burrows when the tide flats are exposed, or when frightened (Barlow, 1961). The special respiratory adaptations of *Gillichthys* have been described by Todd and Ebeling (1966). *Gillichthys* is a facultative aerial breather, gulping air when conditions become unfavorable for aquatic respiration. Gaseous exchange between air bubble and blood occurs by way of the heavily vascularized epithelium of the buccopharyngeal cavity.

Another goby, *Coryphopterus nicholsii* Bean, is less closely related to *Typhlogobius* and does not belong to the *Lepidogobius* species group. In southern California it is found offshore on sand and clay bottoms from less than 10 to over 200 meters

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depth. Oxygen concentrations in these open waters deviate only slightly from air saturation.

The purpose of this investigation was to compare the respiratory, metabolic, and behavioral responses of these three gobiid fishes to axyphia. Since one of these fishes (*Typhlogobius*) dwells throughout its adult life in an environment frequently impoverished with respect to free oxygen, it was expected that respiratory and metabolic adaptations of special interest might be found. Metabolic oxygen requirements were established for each of the species, and abilities to sustain oxidative metabolism at low ambient oxygen tensions were studied. Attention was given to the significance of the oxygen stores under asphyxial conditions, and also to the possible importance of anaerobic glycolysis for prolonging asphyxial survival.

Materials and Methods

All fishes were kept in individual circular specimen jars with a capacity of approximately two liters. Temperature was held at 15 ± 0.5° C and lights were cycled on a twelve hours on- twelve hours off regime by an automatic timer. Fishes were maintained under these conditions for two to eight weeks before use for experimental purposes, and were fed at intervals of two to three days on a variety of fresh and frozen foods.

Respirometry

The respirometer chamber was machined from aluminum and anodized to prevent corrosion. This chamber had a cylindrical bottom portion into which the piston-like lid was inserted, giving a closed volume of 82.9 ml. Two "O" rings on the lid insured a gas tight seal. Oxygen tension was monitored by a polarographic oxygen electrode (Beckman model 777) inserted through an O-ring seal in the lid.

The oxygen electrode was calibrated in air, at 15° C and 100% relative humidity, before every experiment. A constant rate of stirring was maintained by a magnetic stirring bar. Because the bar would not spin reliably beyond a certain rate, it was necessary to stir at a suboptimal rate and apply a corrective factor to the calibration (Cary and Teal, 1965). The calibration correction was determined by calibrating the electrode in air and checking the electrode readout in sea water against the conventional Winkler method over a wide range of oxygen tensions. Once calibrated, the oxygen electrode was quite stable; electrode drift never exceeded 2% of full scale after 24 hours of operation.

The change in oxygen tension in the respirometer chamber was monitored by the oxygen analyzer as it was progressively lowered by respiration of the experimental animal. Changing oxygen tension was recorded on a 5 inch chart recorder (Bausch and Lomb VOM-4). These data, along with the volume of the chamber, the solubility of oxygen in seawater, and the weight of the animal, allowed the rate of oxygen uptake per unit weight of animal to be calculated over any chosen oxygen tension interval. All respirometry was carried out at a temperature of 15 ± 1° C and a salinity of approximately 33.6‰.

Animals were starved for several days before experimental runs so that they would be in a postabsorptive state. Microbial respiration was eliminated by filtering

all seawater through a 0.4 μ glass filter (Millipore) and by adding 100 mg of streptomycin sulfate per liter.

Because excitement and fright of fishes due to handling is known to cause increased activity and an elevated rate of oxygen consumption (Wells, 1932; Fry, 1957), the fish used in this study were protected from outside stimulation in several ways. The interior of the respirometer was darkened, and the respirometer was acoustically insulated by placing a $\frac{1}{2}$ inch felt mat beneath it. From 8 to 12 hours were allowed for diminution of handling excitement and for adjustment to the chamber. A relatively constant rate of oxygen uptake was observed in most cases after this period of time. A flow of aerated, temperature equilibrated seawater was maintained through the respirometer throughout the acclimation period.

Elevated oxygen consumption rates were sometimes noted and were associated with high levels of activity. Although it was not possible to observe fishes in the respirometer, an estimate of activity could be obtained by taking advantage of the sensitivity of the oxygen electrode to stirring. The magnetic stirring motor was switched off and the drop in oxygen tension noted. At very low levels of activity, agitation of the water in the respirometer due to movements of the fish was minimal and the mean indicated oxygen tension would drop to about 50% of the true value. At high levels of activity, the mean indicated oxygen tension would drop only 15–30%, showing many sharp upswings corresponding to individual swimming movements.

Gasbladder sampling

Samples were removed from the gasbladder by body wall puncture and withdrawn into a 1 ml acid-citrate filled syringe. The fish were anesthetized with quinaldine (Eastman Kodak) and restrained underwater during sampling to prevent accidental contamination with air. A portion of the gas sample was transferred to a Scholander microgas analyzer (Scholander, Van Dam, Claff and Kanwisher, 1955) for determination of oxygen, carbon dioxide, and nitrogen (inert gas) content. Duplicate analyses generally agreed within 1 vol% or better.

Reliable estimates of gasbladder volume in intact fish were obtained by pressure-volume manometry (Kanwisher and Ebeling, 1957). Five or six replicate measurements of volume were made for each fish and averaged; replicates usually differed by less than 10%. Blank compliance of the apparatus was approximately 3 μl, so calculated gasbladder volumes were corrected by this volume.

To determine whether anoxic individuals of each of the three species could metabolize gasbladder oxygen, fishes were asphyxiated in sealed syringes for various periods of time before their gasbladders were sampled. Fishes confined in unsealed syringe barrels served as controls, except for several controls which were taken directly from aerated water.

Lactate production and excretion

Asphyxiated fishes were assayed for lactic acid to determine whether energy was being derived from anaerobic glycolysis. Fishes were weighed and 24–48 hours later sealed in flasks or syringes filled with nitrogen-equilibrated seawater.

After a predetermined period of time they were removed, decapitated, and rapidly ground in a mortar with 6 to 24 body volumes of 0.6 M perchloric acid. The ground tissue and perchloric acid were allowed to equilibrate for 1 to 2 hours with occasional stirring; equilibrium was attained in less than 1 hour. After centrifugation the supernatant was assayed for lactate enzymatically, using commercial (Calbiochem or Biochemica) reagents; duplicate analyses using both sets of reagents agreed within 3 mg%. The test reagents contained nicotinamide-adenine dinucleotide (NAD) and lactate dehydrogenase, which reacted with lactate to produce pyruvate and reduced NAD (NADH). The reaction was driven to completion by trapping of pyruvate. Absorption of the NADH formed was measured at 340 mu with a Bausch and Lomb (Spectronic 20) spectrophotometer. Two or more lactate standards of known concentration were run with each set of samples so that net absorptions could be accurately converted to lactate concentrations. Samples were usually determined in duplicate and averaged.

The possibility that *Typhlogobius* might excrete lactate ion either during or following asphyxia was also investigated. Water samples were taken from syringes in which specimens of *Typhlogobius* had been confined with 7 to 10 ml of nitrogen-equilibrated seawater for various periods of time. Samples were also taken from open beakers of aerated seawater in which individuals had been allowed to recover after long periods of asphyxia. All water samples were mixed 1:1 with perchloric acid and assayed for lactate as previously described. Blanks and standards were prepared using seawater.

Asphyxial survival

Survival ability under asphyxial conditions was studied by observing fishes sealed in syringes or flasks of deoxygenated seawater. Deoxygenated seawater was prepared by two different procedures. The first involved heating several liters of seawater to boiling and then cooling rapidly while bubbling with nitrogen. This treatment lowered the oxygen content to nearly zero, and also raised the pH to approximately 8.7 by removing dissolved CO_2. The second procedure was to seal several *Gillichthys* in a flask of seawater for 12–20 hours. Respiration of the confined fishes lowered the oxygen tension of the water to 3 mm Hg or less, and the pH to 7.2–7.3.

Both glass syringes and glass-stoppered flasks of various sizes were used in survival experiments. When flasks were used, they were first inverted in a water bath and the contained water displaced with nitrogen. The nitrogen was then displaced with deoxygenated water. After filling and overflowing the flask, the experimental animal was introduced and the flask sealed. Contamination of syringe water was minimized by flushing with 5–6 volumes of deoxygenated water after placing the animal inside. Oxygen contamination resulting from these procedures was shown by repeated blank determinations to be less than 3 mm Hg.

After an experiment was begun, fishes were observed at frequent intervals. The end point chosen was total cessation of respiratory movements. When inverting the flask or syringe no longer elicited even feeble brachiostegal movements, the animal was removed. Often a water sample was taken from each flask or syringe for determination of final pH (Corning Model 12 pH meter).

Burrow habitat sampling

In order to determine the pH and PO_2 prevailing in the burrows of *Callianassa* during tidal exposure, a piece of polyvinylchloride tubing was pushed into the burrows and water samples were withdrawn into a 10 ml glass syringe. Contamination was avoided by use of a three way syringe stopcock which allowed the dead space to be cleared of air and flushed before filling of the sample syringe. Interstitial water samples were obtained by a sampling device constructed from stainless steel tubing, which was pushed into the substrate to the desired sampling depth.

Sample syringes were sealed with a gastight plug and stored on ice until they could be returned to the laboratory and analyzed, usually less than five hours after collection. Sample oxygen tensions were determined in the laboratory with an oxygen electrode (Radiometer) calibrated against N_2 and air. The absence of significant oxygen tension changes in the iced syringes was confirmed by comparing the oxygen tensions of replicate samples taken from the syringes at intervals of several hours.

Results

Relation between oxygen tension and oxygen uptake rate

Quiescent individuals of each of the three species regulated oxygen uptake over a wide range of oxygen tensions. The four typical plots shown in Figure 1 indicate that oxygen uptake began to decline only after the oxygen tension had fallen to a relatively low level.

In about 20% of the *Typhlogobius* respirometric experiments there was evidence of persistent activity even after 8 to 12 hours of acclimation to the respirometer chamber. The level of activity was frequently estimated by observing indicated oxygen tension readings in the absence of stirring. Active animals produced many rapid fluctuations in the indicated reading, corresponding to individual swimming movements. The concomitant oxygen uptake data invariably indicated a high and fluctuating uptake rate, which usually declined as the oxygen tension in the chamber decreased. Such an "oxygen-dependent" respiratory pattern has been described in various fish species, and Beamish (1964a, 1964b) has demonstrated a correlation between oxygen dependence and muscular activity in several species of fish. In the present study, data showing a strongly fluctuating or steeply declining oxygen uptake rate due to activity were discarded.

In order to characterize the tension range associated with loss of ability to regulate resting oxygen uptake, it was determined within which 2% saturation interval uptake first fell to 85% or less of the resting rate determined for that individual fish. The *preceding* (higher) 2% interval was designated as the critical oxygen tension interval. The 85% definition was arbitrarily chosen, but the break in slope was usually sharp, and an 80% or 90% criterion would have had little effect on the critical levels established for each of the species. The critical interval for each plot in Figure 1 is indicated by an arrow.

Critical tensions determined for individuals of a species fell within a limited range, as shown in Table I. Disregarding the single highest value for each species, the range of critical oxygen tensions was 6–10% saturation (9–16 mm Hg) for *Typhlogobius*, 10–16% saturation (16–25 mm Hg) for *Gillichthys*, and 12–18% (19–28

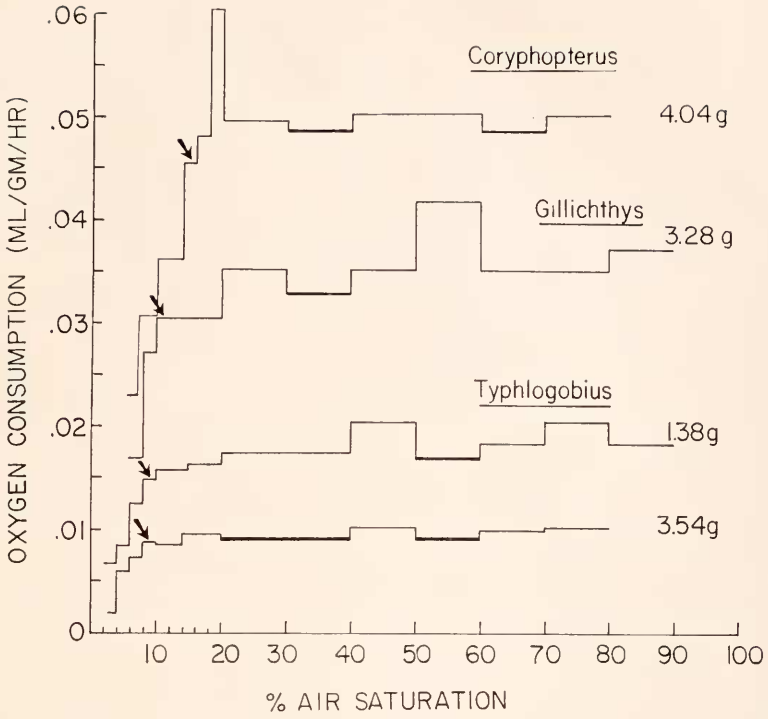

FIGURE 1. Four typical plots showing oxygen consumption at 15° C as a function of oxygen tension. The heavy lines indicate resting oxygen consumption rates and the arrows designate the tension at which oxygen consumption becomes tension-dependent (critical level). Wet body weights are given for each example.

mm Hg) for *Coryphopterus*. Critical oxygen tension distributions were compared by the Neuman-Keuls analysis of variance (Sokal and Rohlf, 1969) and found to differ significantly at the 0.01 probability level in all comparisons.

The Kruskal-Wallis sum of ranks test (Tate and Clelland, 1957) was used to test for possible correlation between weight and critical interval within species. No correlation was indicated ($P > 0.1$ in each case).

TABLE I

Distribution of critical oxygen tension intervals (1% saturation = 1.56 mm Hg).

	N	Critical intervals (% Saturation)						
		6–8%	8–10%	10–12%	12–14%	14–16%	16–18%	18–20%
Typhlogobisu	13	4	8	1				
Gillichthys	15			6	5	3	1	
Coryphopterus	11				4	3	3	1

Resting oxygen consumption

For estimation of the resting or "standard" metabolic rate, the rate of oxygen uptake was calculated for each 10% saturation interval down to and including the 40–30% interval (which was safely above the range where oxygen uptake became tension dependent and began to decline). The lowest uptake rate calculated for any 10% saturation interval was taken as the resting rate. This procedure is similar to that followed by Poulson (1963) and Job (1955) who made serial determinations of oxygen uptake after a predetermined calming period, and recorded the lowest as the standard metabolic rate. The presumed resting (standard) oxygen consumption rate is indicated by a heavy line in each of the four examples of Figure 1.

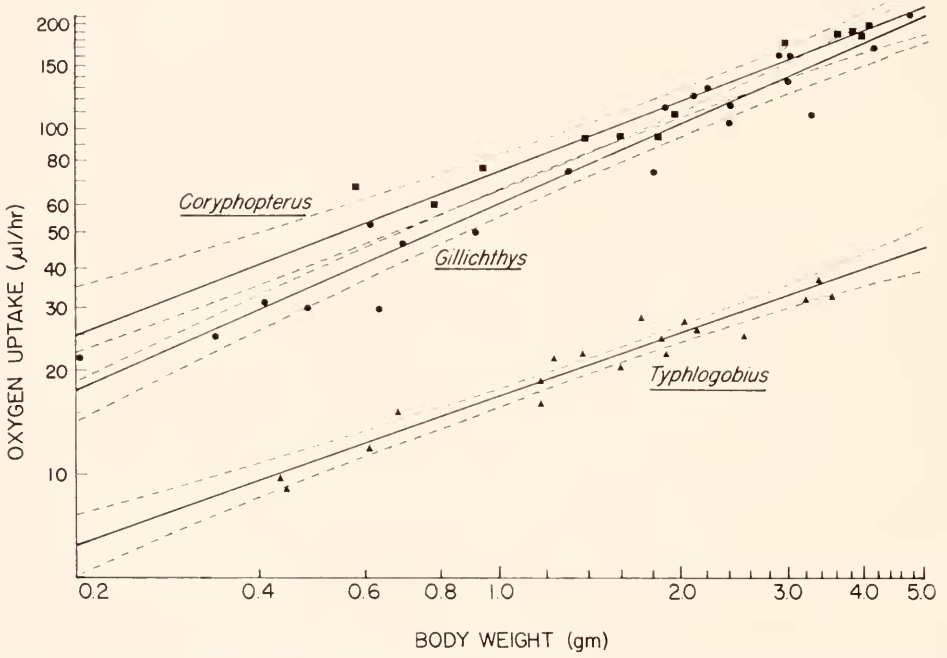

FIGURE 2. The relation between log wet body weight and log resting oxygen consumption for *Typhlogobius, Gillichthys,* and *Coryphopterus,* with 95% confidence limits.

Relation between weight and resting oxygen consumption

Regression of the logarithm of resting oxygen consumption per total weight on the logarithm of wet weight was calculated by the Bartlett (1949) procedure (Fig. 2). Linearity tests confirmed a good fit to a straight line for each of the three species. Regressions of log uptake/unit weight on log wet weight tended to be curvilinear (Marr, 1955).

In every case regression coefficients were less than one, indicating that resting oxygen consumption was not linearly related to wet body weight (Table II). Comparison of slopes by a modification of the Scheffe multiple comparison test (Scheffe, 1959) indicated there were no signficant differences. However, the regressions

TABLE II

The relation between resting oxygen uptake and wet body weight. The regression equation $\log M = \log a + b \log Wt$; *M is* $\mu l\ O_2/hr$ *and Wt is grams*

	N	a	95% C.L. for a	b	95% C.L. for b
Typhlogobius	18	16.6	15.7–17.4	0.615	0.521–0.716
Gillichthys	20	59.0	54.1–64.3	0.762	0.657–0.871
Coryphopterus	13	74.0	68.4–79.8	0.676	0.551–0.825

did differ significantly in position as indicated by a comparison of the 95% confidence limits for mean predicted oxygen uptake in Figure 2. It is evident that *Typhlogobius* consumes oxygen at a much lower rate than either of the other two species.

Utilization of gasbladder oxygen

Gasbladder volumes determined by volume-pressure manometry for six individuals of each species and expressed as ml gas/100 g were as follows ($\bar{x} \pm$ S.D.): *Typhlogobius*, 3.9 ± 0.2; *Gillichthys*, 3.2 ± 0.9; and *Coryphopterus*, 3.5 ± 1.06. Volumes of *Typhlogobius* gasbladders ranged from 3.6 to 4.1 ml/100 g. Data for the two other species were more variable; high values were close to or within the *Typhlogobius* volume range, but several individuals apparently had partially deflated gasbladders.

The composition of gases in the gasbladder was also quite variable (Table III). *Gillichthys* gasbladder oxygen ranged from 3.4 to 53 vol%, even though all animals had been resting quietly in well aerated seawater for at least 10 days prior to sampling.

Both *Typhlogobius* and *Gillichthys* utilized gasbladder oxygen rapidly under asphyxial conditions (Table IV). Oxygen in the gasbladder of *Typhlogobius* had declined to less than 2 vol% after 2 hours of asphyxia and to less than 1 vol% after 4 hours. *Gillichthys* gasbladder oxygen had declined to 3 vol% or less after 1.5 to 2.5 hours of asphyxia. The limited accuracy of the analytical procedure made it impossible to determine whether gasbladder oxygen was eventually completely utilized.

Typhlogobius gasbladders sampled in the field were not depleted of oxygen. Five individuals were collected on a −2.2 foot low tide in January, 1969. They

TABLE III

Gasbladder O_2 *and* CO_2

	N	O_2 (vol %)		CO_2 (vol %)	
		$\bar{X} \pm$ S.D.	Range	$\bar{X} \pm$ S.D.	Range
Typhlogobius	7	29.3 ± 18	12–67%	0.6 ± 0.4	0–1.0%
Gillichthys	18	24.8 ± 17	3–53%	1.1 ± 0.7	0–2.6%
Coryphopterus	7	34.1 ± 17	20–51%	1.2 ± 0.9	0–2.8%

were sampled at intervals of 20–30 minutes, beginning one hour before low water, and ending one hour after. The percentage oxygen in the gasbladders of these fishes ranged from 18.1% to 27.4% (24.6%, 18.1%, 27.4%, 22.2%, 25.4%) with a mean of 23.5%, agreeing reasonably well with the mean 26.4% oxygen found in gasbladders of 11 laboratory acclimated animals (Tables III and IV).

The mean oxygen in gasbladders of four specimens of *Coryphopterus* which succumbed to asphyxia was somewhat lower (23.8 ± 14 vol%) than mean oxygen in gasbladders of aerobic controls (34.1 ± 15 vol%), but the difference was not significant ($P < 0.20$; Mann-Whitney U test). It is apparent that *Coryphopterus* does not use the gasbladder as an emergency oxygen store to any important degree.

Lactate production and excretion

Accumulated lactate was expressed as μM per gram of wet weight. Since fishes of differing weights were used in these experiments, the data were corrected for the effect of weight on metabolic rate. It was assumed that the relation established

TABLE IV

Utilization of gasbladder oxygen under asphyxial conditions

Typhlogobius			Gillichthys		
Time	n	$\%O_2$ remaining	Time	n	$\%O_2$ remaining
Control	4	21.4	Control	4	35.0
1 hr	1	4.4	10 m	1	43.0
2 hr	1	1.3	20 m	2	20.2
3 hr	1	1.0	30 m	1	14.0
4 hr	1	0.9	1 hr	3	11.0
6 hr	1	0.3	1 hr 30 m	2	2.2
21.5 hr	1	0.3	2 hr	2	1.7
25.5 hr	1	0.0	2 hr 30 m	1	0.3

between weight and aerobic metabolism rate ($M = aWt$, Table II) could be extended to describe the relation between weight and anaerobic metabolic rate. Correction was made to the mean weight of all fish of a species used in this experiment ($\bar{x}$ weight *Typhlogobius* = 1.5 g; $\bar{x}$ weight *Gillichthys* = 2.5 g). The lactate concentration found in an x gram *Typhlogobius* was multiplied by the corrective factor M/Wt (1.5 g fish) / M/Wt (x g fish); M is the calculated resting total oxygen uptake (Table II). This correction altered most lactate values by less than 15%.

A linear regression equation of corrected *Typhlogobius* lactate values *versus* duration of asphyxia was fitted by the least squares procedure. The significant fit (linearity t test) of the data indicated a steady rate of lactate accumulation up to and presumably beyond the forty-sixth hour. The regression slope was 1.16 μM/g/hr with 95% confidence limits of 0.90 to 1.41 μM/g/hr.

The *Gillichthys* lactate accumulation data were much more variable than the *Typhlogobius* data, perhaps as a result of the higher metabolic rate and more active behavior. The least squares regression slope was 3.3 μM/g/hr with broad 95% confidence limits overlapping zero (−0.96 to 6.9 μM/g/hr).

There was no indication that *Typhlogobius* could excrete lactate ion either during asphyxia or recovery from asphyxia. None of the seawater samples assayed for lactate deviated from zero by more than ±0.3 μM/l, approximating the limits of precision for the analytical procedure.

Asphyxial survival

Specimens of *Typhlogobius* and *Gillichthys* initially responded to deoxygenated water by increasing ventilation rate and volume, but this phase was brief and soon followed by a pronounced slowing or complete cessation of ventilation. Eventually both species resumed respiratory movements at a low amplitude and rate. Respira-

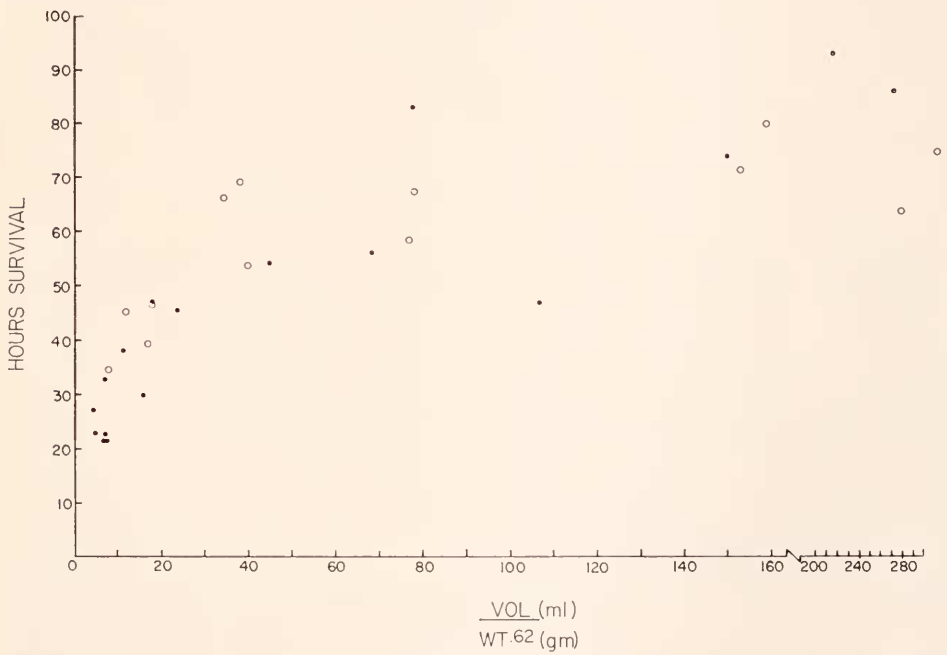

FIGURE 3. Relation between asphyxial survival of *Typhlogobius* and seawater volume/wet body weight ratio (corrected for metabolic rate). Closed circles indicate an initial seawater pH of 7.2 to 7.4; open circles an initial pH of 8.7. The initial P_{O_2} was always less than 3 mm Hg.

tory movements gradually became increasingly irregular and infrequent and finally ceased entirely.

Coryphopterus responded quite differently to deoxygenated water. The initial ventilatory response was a series of convulsive "coughs." The fish then began to swim violently upward, continuing until exhaustion curtailed further effort.

The time taken for respiratory movements of asphyxiated specimens of *Typhlogobius* to fail ("survival time") was found to vary with the volume of enclosing seawater. This is shown in Figure 3 by the plotting of survival time against

the ratio seawater volume(ml)/wet body weight(g)$^{0.62}$. The 0.62 power of the body weight was used since it had been determined by respirometry to bear a proportional relationship to metabolic rate (Table II), and it was felt that asphyxial survival time should be more closely correlated with the inverse of metabolic rate than with the inverse of body weight. The ratio seawater volume (ml)/wet body weight (g)$^{0.62}$ will be referred to as "relative volume." The data points of Figure 7 may be fitted by an exponential regression equation of the form $x = pe^{ky}$. Rewritten with y (hours survival) as the dependent variable, and replacing x (volume/Wt$^{0.62}$) with logarithm x, the relationship becomes linear: $y = -0.796 + 14.946 \ln x$ (Bartlett regression). A linearity t test indicated that this regression provides a good fit to the data points. The 95% confidence intervals were ±2.94 for the slope and ±3.53 for the y intercept.

The survival of asphyxiated specimens of *Gillichthys* was independent of the relative volume of enclosing seawater. Survival times ranged from 6.5 to 12.5 hours for well nourished animals acclimated for one month. Another group which had been kept in the laboratory for four to five months and fed at irregular intervals survived only 1.5 to 4.5 hours of asphyxia.

Coryphopterus survival also was independent of volume. Survival times in nitrogen-equilibrated seawater ranged from 28 to 42 minutes.

Although cessation of ventilation was selected as a convenient end point for survival experiments, the animals were not necessarily dead when ventilation ceased. *Typhlogobius* and *Gillichthys* could be revived by removal to fresh seawater after no signs of consciousness or life had been apparent for some time. *Typhlogobius* often recovered after several hours in this state, although recovery following long periods of asphyxia was quite protracted. Often animals in the eighth hour of recovery or later would still not have regained equilibrium, and would be hyperventilating rapidly. However, no lasting effects could be detected. Animals revived after 80–90 hours of asphyxia took food the following day and lived for months afterwards in the laboratory.

In contrast, irreversible changes apparently occurred in the central nervous system of *Coryphopterus* a few minutes following stoppage of respiration. These changes were made manifest initially by twitching or quivering of the gill archs and pectoral fins. Shortly afterwards the dorsal and anal fins began to quiver and occasionally the trunk muscles would perk sharply. These convulsive movements lasted for 15–20 minutes, and the animals could not be revived once they had begun.

The initial carbon dioxide content of deoxygenated seawater did not seem to have any effect on the survival of *Typhlogobius*. The open circles in Figure 3 represent survival of animals in nitrogen-equilibrated seawater with an initial pH of 8.7; closed circles represent survival of animals in *Gillichthys*-preconditioned seawater with an initial pH of 7.2–7.3.

The pH of seawater contained in syringes and flasks was lowered by the metabolism of enclosed specimens of *Typhlogobius*. The terminal pH at cessation of respiration was related to the initial pH and to the relative volume of seawater. At lower relative volumes (< 10) a minimum pH of approximately 5.4 was attained, regardless of the initial pH. The inflection point in the asphyxial survival curve (Fig. 3) at a relative volume of approximately 25–35 coincides with a terminal pH of approximately 5.7–5.8; this relationship is more clearly shown by Figure 4.

FIGURE 4. Correspondence between survival time of asphyxiated *Typhlogobius* and terminal seawater pH. Closed circles indicate an initial pH of 7.2–7.4; open circles an initial pH of 8.7

The decrease in seawater pH was in part due to excretion of CO_2. However, because of the limited amount of oxygen available in the gasbladder, only a small amount of CO_2 could have been produced by oxidative metabolism. With a respiratory quotient of unity, the oxygen contained in the gasbladder of a 1.5 g *Typhlogobius* (4% body volume; 25 vol% O_2) could produce about 0.7×10^{-3} mM of CO_2. Additional CO_2 would have been produced by bicarbonate buffering of anaerobically produced lactic and pyruvic acids. If *Typhlogobius* has large bicarbonate reserves in the blood comparable to those of the electric eel *Electrophorus* (30 mM/l; Johansen, Lenfant, Schmidt-Nielsen and Petersen, 1968), and if 5% of the body weight is blood, the blood would contain 2.3×10^{-3} mM bicarbonate that could be converted to CO_2. The total amount of metabolic CO_2 and buffer-produced CO_2 (3×10^{-3} mM) would lower the pH of 20 ml of seawater by about 0.4 pH units (Strickland and Parsons, 1965). A 1.5 g *Typhlogobius* confined in 20 ml of seawater (relative volume = 16) actually lowered the pH by 2–3 units.

Additional evidence indicated that carbon dioxide was not the only acid substance excreted by *Typhlogobius* during asphyxia. The presence of a non-volatile acid was shown by the observation that asphyxial seawater could not be returned to the pH of normal air-equilibrated seawater (8.0–8.2) by bubbling with air. Aeration of seawater with a terminal pH of 5.4 raised the pH to approximately 7.5. No further change in pH occurred after the first hour of aeration, even when bubbling was continued for eight hours or more. Control trials with CO_2-saturated water indicated that one-half hour of aeration was sufficient to wash out all dissolved CO_2.

It may be hypothesized that an acid byproduct of anaerobic metabolism would, if excreted at a constant rate, attain a concentration proportional to the survival

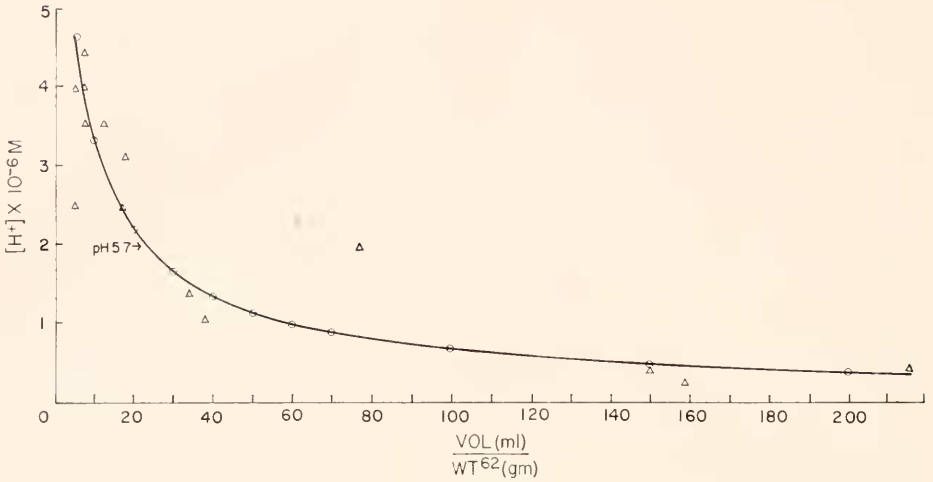

FIGURE 5. Terminal concentration of excreted metabolic byproducts following asphyxiation of *Typhlogobius* in different volumes of seawater. Circles represent the calculated concentration (arbitrary units) of a byproduct excreted at a constant rate throughout the period of asphyxiation. Triangles represent actual measured hydrogen ion concentrations.

time and inversely proportional to the relative volume ($Vol/Wt^{0.62}$). An equation relating survival time to relative volume has previously been derived, so the terminal byproduct concentration may be expressed:

$$\text{Conc.} = k\,\frac{-0.796 + 14.95(\text{Vol } Wt^{0.62})}{\text{Vol } Wt^{0.62}}$$

where k is a constant. If this expression is plotted in arbitrary units against relative volume (represented by the plotted curve in Figure 5) it may be seen that the estimated byproduct concentration begins to increase rapidly below a relative volume of about 25 to 35. Plotting of actual measured hydrogen ion concentration (terminal pH) against relative volume in Figure 5 shows close agreement with this expression; hydrogen ion concentration is seen to increase rapidly below a relative volume of 25 to 35, corresponding to a pH of 5.7 to 5.8. Figures 3 and 4 indicate that survival time falls off rapidly below relative volumes of 25 to 35 and below a pH of 5.7 to 5.8. This suggests that the exponential decrease in asphyxial survival time of *Typhlogobius* with decreasing seawater volume may result from accumulation of an excreted acid metabolite.

Sampling of burrow habitat

The area selected for sampling of *Callianassa affinis* burrow water and interstitial water was a shingle and sand beach at Point Loma, California. Most individuals of *Typhlogobius californiensis* used in this study were collected in the immediate area. The sampling site was at an elevation of 25 to 30 cm above Mean Lower Low Water and remained exposed for 5.5 hours on a −1.5 foot low tide.

Six interstitial water samples were taken at 15 minute intervals from a depth of 20 cm in the substrate while the sampling area was still 5 to 25 cm awash. Oxygen tensions were 3.0, 0.8, 3.8, 6.1, and 3.5 mm Hg. A transient increase in interstitial oxygen tension was observed as the surface drained; this may have been caused by a downward percolation of surface water. Successive samples showed a rapid decline in oxygen tension. In two hours the oxygen tension fell to 4 mm Hg, followed by a further slow decrease to about 1 mm Hg after four hours.

The oxygen tension of water from burrows of *Callianassa* differed little from the oxygen tension of interstitial water, declining at the same rate to the same low levels. Four hours after tidal exposure samples from a depth of 15 to 18 cm in two burrows of *Callianassa* had oxygen tensions of 0.5 and 3 mm Hg.

Discussion

Many burrowing invertebrate animals are suspension feeders, creating a current through the burrow which carries in particles of food from the outside. *Callianassa affinis,* the shrimp that serves as host for *Typhlogobius californiensis,* causes a feeding current to flow through its burrow by the action of the abdominal swimmerets (MacGinitie, 1939). The entrances to *Callianassa affinis* burrows are submerged for a major portion of every day (18 to 24 hours), and the frequently renewed water in the burorws remain relatively well oxygenated during this time. In the interstitial water outside the burrows low oxygen tensions and pHs generally prevail, due to the limited rate of exchange with the overlying oxygen-rich water.

Low minus tides may expose the burrows of *Callianassa* for 5 to 6 hours. Circulation of water through the burrows is interrupted during the period of exposure, and oxygen tensions in the deeper portions rapidly equilibrate with the interstitial water, falling to almost anoxic levels. However, some diffusive exchange with the air can occur at the burrow entrance, the rate of exchange being severely limited by the small interface area. Farley and Case (1968) have shown that *C. affinis* responds to low oxygen tensions with an increase in ventilatory rate. Under natural conditions acceleration of ventilatory activity would mix oxygenated water deeper into the burrow and enhance the rate of invasion of oxygen.

A marked tendency for specimens of *Typhlogobius* to move into a flow of hypoxic water was observed in laboratory experiments (unpublished observations). In the field this response to hypoxia should result in the goby moving upward in the burrow to a position near its hyperventilating host *Callianassa.* The water in the upper part of the burrow need be only partially oxygenated (9–12 mm Hg) for *Typhlogobius* to satisfy its meager oxygen requirements.

The fact that none of the *Typhlogobius* gasbladders sampled in the field were depleted of oxygen also argues that the fish ordinarily avoid asphyxial conditions. Induced asphyxia in the laboratory resulted in almost complete utilization of gasbladder oxygen within 1 to 2 hours.

Callianassa affinis burrows could not be expected to always remain open to the surface. The habitat is exposed to the open sea, and even the large rocks which ordinarily shelter the burrows are sometimes shifted about by the action if unusually powerful waves. At such times many burrow entrances must be blocked or filled in. If denied access to the surface, *Typhlogobius* would be unable to escape the hypoxic

conditions prevailing in the substrate, and would have to rely largely upon anaerobic metabolism until the host shrimp could open a new entrance. Because the reproductive potential of these long-lived fishes is spread over a period of many years (MacGinitie, 1939), such circumstances could occur infrequently and still be strongly selective for anaerobic competence.

The relations established between body weight and oxygen consumption for the species studied are comparable to those found for other species by other investigators. The regression of log oxygen consumption against log weight generally is linear with a slope between 0.70 and 0.90 (*cf.* Fry, 1957; Beamish, 1964a). The log rate-log weight regression slope of 0.615 found for *Typhlogobius* is somewhat lower than most reported slope values, and may be of adaptive advantage in reducing the oxygen requirements of larger individuals.

Typhlogobius has a very low rate of resting oxygen consumption, only 20–30% that of *Gillichthys* and *Coryphopterus*. *Typhlogobius* oxygen requirements are also much smaller than most oxygen uptake rates reported in the literature for inactive fishes (Beamish, 1964b). The depressed metabolic rate of *Typhlogobius* may serve to bring food and oxygen requirements into balance with limited supplies available in the habitat. A low metabolic level would also conserve energy substrate stores during periods of protracted anaerobiosis.

Typhlogobius, Gillichthys and Coryphopterus regulate oxygen consumption down to oxygen tensions of 9–16, 16–25, and 19–28 mm Hg, respectively, at 15° C. The critical oxygen tensions found for these gobies support the generalization that ability to regulate oxygen consumption is correlated with oxygen availability in the habitat; animals living in periodically or chronically stagnant waters, such as *Typhlogobius,* usually have lower critical oxygen tensions than those living in well aerated waters (Prosser, 1955). However, even *Coryphopterus* regulates to a relatively low tension in comparison with many other fishes. It is interesting that the critical oxygen tension range found for *Typhlogobius* (9–16 mm Hg) is in close agreement with the critical oxygen tension range established for *Callianassa affinis* (10–20 mm Hg) by Thompson and Pritchard (1969).

Under asphyxial conditions both *Typhlogobius* and *Gillichthys* rapidly metabolize gasbladder oxygen, reducing the oxygen concentration to 1 vol% or less within 2–3 hours. Gasbladders of each of the species studied had filled volumes of about 4 ml per 100 g body weight. Oxygen concentrations averaged 25–35 vol%, so the quantity of oxygen contained in the gasbladder of a typical 1 g fish would be about 10 μl (4% body volume; 25 vol% O_2). Calculated on the basis of the oxygen uptake rates established by respirometry, reasorbed oxygen would have been sufficient to meet approximately one-half of the oxygen requirements of *Typhlogobius* and one-fifth or less of the requirements of *Gillichthys* during the first hour of asphysia. Considering the limited probable quantity of the other oxygen stores in the blood and tissue fluids, it is unlikely that the blood could retain any appreciable amount of oxygen beyond the third or fourth hour of asphyxia.

Many fishes are known to resorb oxygen from the gasbladder when asphyxiated (Jones, 1957). However, the respiratory significance of this oxygen has been questioned. Black (1942) expressed the opinion that respiratory benefit to the tissues was unlikely, because in euphysoclists blood from the gasbladder returns directly to the heart and is mixed with deoxygenated venous blood. Steen (1963)

pointed out that this mixed blood must pass through the gills before reaching the tissues, and that oxygen would diffuse from partially oxygenated blood to oxygen-free water. A diffusive loss of oxygen could be prevented by not perfusing the gills or by shunting the blood away from the gill lamellae (Fange, 1966). This may be why *Typhlogobius* and *Gillichthys* immediately slow or stop gill ventilation when exposed to anoxic water, although energy conservation would be an important additional benefit. Non-respiratory shunts have been found in the gill filaments (Steen and Kruyse, 1964), but their function in asphyxia has not been investigated.

Although the blood of *Gillichthys* and *Typhlogobius* is almost certainly anoxic after the third or fourth of asphyxia, both species tolerate asphyxia for much longer periods. *Gillichthys* survives for 6.5 to 12.5 hours in anoxic seawater, while *Typhlogobius* survives for up to 60–94 hours. During this time maintenance energy must be derived from anaerobic metabolism.

Accumulation of lactate by anoxic specimens of *Typhlogobius* indicates that energy is produced by anaerobic glycolysis. Each μM of glucose-1-phosphate (from glycogen) which is fermented to 2 μM of lactic acid produces 3 μM of adenosine triphosphate (ATP). Under aerobic conditions, oxidation of one μM of glucose-1-phosphate produces 39 μM of ATP and utilizes 5 μM of oxygen. Therefore, anaerobic production of 1μM of lactate is energetically equivalent to the consumption of 0.23 μM, or 5.2 μl, of oxygen in aerobic respiration. The 95% confidence limits for the slope of the lactate accumulation regression are 0.90 to 1.41 μM/g/hr. The resting oxygen uptake rate of a 1.5 g *Typhlogobius* is 14 μl/g/hr, so 95% confidence limits for anaerobic energy production are equivalent to 33–52% of the aerobic rate.

If lactate production by *Typhlogobius* corresponds quantitatively to glycogen utilization, then the overall anaerobic energy metabolism is reduced to half or less of the aerobic metabolism. It is known that energy metabolism is reduced in diving birds and mammals, probably as a result of mass-action blocking of metabolic pathways in uncirculated tissues (Scholander, 1964). The possibility also exists that end-products of anaerobic metabolism other than lactate may be formed. Various acid end-products, such as acetic, proprionic, and succinic acids are produced by the anaerobic metabolism of molluses (Mehlman and von Brand, 1951; Hammen, 1969), while roundworms (Saz and Weil, 1960) channel glycogen into fatty acids. Although these or other processes may play a minor role in the anaerobic metabolism of *Typhlogobius,* it is probable that production of lactic acid is the dominant energy-yielding process.

Oxygen is generally considered essential for maintenance of life in vertebrates. While some tissues tolerate anoxia relatively well, other tissues are rapidly damaged or killed. The brain is most vulnerable in this respect. The vulnerability of the vertebrate brain to anoxia is related to the high energy requirements of nerve cells, and energy requirements are correlated with the maintenance of steep ionic gradients across nerve cell membranes (Robin, Vester, Murdaugh and Millen, 1964). Maintenance energy is ordinarily derived by oxidative phosphorylation, as evidenced by the high oxygen consumption of brain tissue, and by the rapid loss of function when oxygen is denied. In some cases it is possible for nerve cells to preserve excitability in the absence of oxygen, if the enzymes of anaerobic glycolysis are present in adequate concentrations, and if an adequate supply of glucose is available (Lampert,

1961). Verzhbinskaya (1952) has pointed out that the brain tissue of fishes contains higher concentrations of glycolytic enzymes than the brain tissue of other vertebrate groups.

The brain of a few vertebrates is apparently capable of extended anaerobiosis. Belkin (1963; 1968) found that some species of turtle would continue to breathe nitrogen for 12 to 24 hours or longer. Foetal and newborn mammals of some species also have a remarkable anaerobic capability (Dawes, 1968). Anoxic survival time of both turtles and newborn mammals is drastically shortened by iodoacetate poisoning, demonstrating the importance of anaerobic glycolysis (Belkin, 1962; Himwich, Berstein, Herrlich, Chester and Fazekas, 1942). A continued exchange of metabolites, presumably glucose and lactic acid, between circulating blood and brain is presumed to be vital for extended anaerobic survival (Belkin, 1968; Dawes, 1968).

The central nervous system of *Typhlogobius* may be especially well-suited to function anaerobically because of its relative simplicity. The brain is proportionally quite small, even in comparison with the small brain of *Gillichthys*. The reduced size of the brain is probably correlated with the overall poor development of sensory modalities (Ritter, 1893; MacGinitie, 1939). Histological examination indicates a poor development of neuronal layers, nuclei and fiber tracts (Scheich, Honnegger, Warrell, and Kennedy, 1973). Electrical activity is low; EEG activity can not be recorded, and evoked potentials are barely detectable by special amplifying techniques (H. Scheich, personal communication). Expenditure of energy for maintenance of ionic gradients must be correspondingly reduced.

A recent study compared the cross-sectional area of open capillaries in the brain of rapidly frozen anoxic and normoxic *Typhlogobius* (Scheich *et al.*, 1972). An extremely rich capillary bed was observed in stained brain sections. The area of open capillaries in anoxic fish averaged 2.4 times greater than in normoxic controls, due primarily to an increase in mean capillary diameter. The resulting five fold increase in capillary surface area would greatly facilitate the exchange of metabolites between blood and brain tissue, and may represent an important adaptation for anaerobic brain metabolism.

Extended anaerobiosis requires not only energy production for maintenance of vital functions, but also some means of counteracting the rise in hydrogen ion concentration resulting from accumulation of lactic and pyruvic acids or other acid metabolites (Beadle, 1961). Turtles, which have large buffer stores, apparently buffer anaerobically generated hydrogen ion (Belkin, 1963; Robin, Vester, Murdaugh, and Millen, 1964). Fishes, which constantly exchange ions and metabolites with surrounding water, may be better able to eliminate excess hydrogen ion. *Typhlogobius* excretes some non-volatile acid product of anaerobic metabolism, but retains lactate ion. Since hydrogen ion and lactate ions are produced in an equimolar ratio, uncompensated loss of H^+ would soon result in an electrochemical imbalance. Evidence has been provided for NH^+_4/Na^+ and HCO^-_3/Cl^- exchange by teleost gills (Maetz and Garcia Romeu, 1964); these processes result in a net exchange of H^+ across the gills. In addition, a H^+/Na^+ exchange system (Garcia Romeu, Salibian, and Pezzani-Hernandez, 1969) has been suggested in frogs. Ionic exchange may permit *Typhlogobius* to eliminate hydrogen ion while preserving blood electroneutrality.

The available evidence suggests that survival of *Typhlogobius* in small volumes of deoxygeneated seawater is curtailed by accumulation of an excreted acid metabolite, acute toxicity becoming evident at a hydrogen ion concentration of about 1.5×10^{-6} molar (pH 5.8). In larger volumes of seawater survival is ultimately limited by some other factor, possibly eventual depletion of the carbohydrate reserves necessary for sustenance of anaerobic glycolysis.

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Summary

1. The respiratory, metabolic, and behavioral responses to asphyxia of three gobiid fishes are compared. Adult specimens of *Typhlogobius californiensis* and *Gillichthys mirabilis* are subject to hypoxic stress in their natural habitats, while *Coryphopterus nicholsii* generally is not.

2. Oxygen tensions in burrows of the ghost shrimp *Callianassa affinis*, commensal host of *Typhlogobius*, fall rapidly to nearly anoxic levels following exposure of the burrow openings by the outgoing tide.

3. The resting oxygen consumption of *Typhlogobius* is extremely low; the one gram intercept for the wet weight-oxygen uptake regression is 17 μl O_2/g/hr. The other two species consume oxygen 3 to 5 times as rapidly at rest.

4. Resting specimens of *Typhlogobius* are capable of maintaining a constant rate of oxygen uptake down to a critical ambient oxygen tension of 9–16 mm Hg. *Gillichthys* and *Coryphopterus* are capable of regulating oxygen uptake down to critical levels of 16–25 mm Hg and 19–28 mm Hg respectively.

5. Both *Typhlogobius* and *Gillichthys* metabolize gasbladder oxygen during the initial few hours of asphyxia.

6. *Typhlogobius* accumulates lactate ion during periods of prolonged asphyxia, indicating that maintenance energy is derived from anaerobic metabolism. The 95% confidence limits for the rate of lactate accumulation are energetically equivalent to 33% to 52% of the respirometrically measured aerobic metabolic rate.

7. The survival time of *Typhlogobius* in deoxygenated seawater varies with the volume of seawater. In very small volumes of seawater ($<$ 10–20 ml), survival time is less than 24 hours. In larger volumes of seawater, survival time asymptotically approaches 80 to 100 hours. The decreased asphyxial survival time in small volumes of seawater may result from rapid accumulation of an excreted acid byproduct of anaerobic metabolism.

Gillichthys survives exposure to deoxygenated seawater for a relatively short period of time; survival times varied from 6.5 to 12.5 hours and were independent of seawater volume. *Coryphopterus* survives anoxia only 28 to 42 minutes.

8. It is hypothesized that the ability of *Typhlogobius* to function as a facultative anaerobe may be related to the low energy demand of the brain, to vascular adapta-

tions which facilitate the exchange of metabolites between brain and circulating blood, and to an ability to excrete the acid by-product(s) of anaerobic metabolism.

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